The Journal of Animal & Plant Sciences, 26(4): 2016, Page: 1056-1062
ISSN: 1018-7081

A FOLIAR APPLICATION SILICON ENCHANCES DROUGHT TOLERANCE IN FENNEL

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ABSTRACT

The objectives of this study were to evaluate improving the drought toleration by the function of Silicon (Si) in fennel. The experimental design was a split-plot with three irrigation treatments: irrigation at 90, 75 and 60% of FC as main treatments, and five levels of Si foliar sprays (0, 2.5, 5, 7.5 and 10 mM) comprising the sub-treatments that were exerted with three replicates. The experiment was carried out during 2014 at the Zabol University Research Farm, Zabol, Iran. Water stress significantly decreased LAI, plant height, one-thousand seed weight, seed yield, concentrations of calcium and potassium in seed, while stimulated concentration of proline, soluble sugars and sodium in seed, as well as concentration of Si in leaf. Si application increased concentration of proline and soluble sugars in seed, LAI, seed yield, essentialoil percentage, concentration of sodium andpotassium in seed by 24, 13, 20, 17, 31, 41, 24 and 9%, respectively, over those of the control. The greatest essential oil percentage (2.17%) was achieved in plant sprayed with 7.5 mM of Si under mild water stress. These results suggested that Si spraying improved growth and physiological indices hence could increase the ability of plants to resistance water stress.

Keywords: Essential oil, Proline, Potassium, Yield and yield attributes.

INTRODUCTION

Drought is one of the most confining factors for crop production worldwide (Ashraf, 2009). Accumulation of osmoregulation is an efficient mechanism in drought-tolerant plants for maintaining turgidity under water stress conditions. Osmoregulation occurs due to accumulation of organic (proline and soluble sugars) and mineral (sodium) solute in cytosol (Good and Zaplachiniski, 1994). Moreover, drought could reduce the absorption of nutrients through changing the osmotic potential of the soil and its effect on water and mineral absorption by the roots (Hu *et al*., 2007). Proper use of plants mineral nutrition is an important strategy to reduce the adverse effects of drought.

Silicon (Si) is the second most abundant element existing on earth (Silva *et al.,* 2012). Although it is not considered as an essential element, nevertheless, there is increasing evidence regarding its positive effects on plant growth and development (Karmollachaab *et al.,* 2014). Si acts as a mechanical or physical obstacle in plants and not only acts as cell wall strengthening, but is also actively involved in many physiological processes. Studies have displayed that positive impacts of Si are more prominent under stressful conditions as it can increase plant defense systems against low (Epstein, 1999) and high temperature (Hattori *et al.*, 2005), UV-radiation (Shen *et al.*, 2010), salinity (Biel *et al.,* 2008) and heavy metal toxicity (Shi *et al.,* 2005). Si is also found to enhance water stress tolerance in plants by retaining leaf water potential, leaves erectness, stomatal conductance, structure of xylem vessels under high transpiration rates, and photosynthetic activity (Gong *et al.*, 2003). Si can diminish the electrolyte leakage from plant leaves and therefore raised photosynthetic activity in plants grown under water stress conditions (Epstein, 1999). Gao *et al.* (2006) suggested that Si influences stomata movement and, therefore, affects transpiration rate through stomata. Gong *et al.* (2003) reported greater water use efficiency by application of Si in wheat. Matoh *et al.* (1991) found that application of Si led to formation of a silica cuticle layer on epidermal tissue of leaf, which is responsible for greater leaf water potential under water deficit conditions.

Fennel (*Foeniculum vulgar* Mill) is one the most widespread medicinal plants of the Apiaceae family. This medicinal plant mainly cultivated with the aim of essential oil production. Water stress is known to limit the growth of medicinal plants and can cause severe decline in their quality (Moosavi *et al.*, 2014). Despite the extensive study on role of Si regarding plant water relations in different field crops under stressful conditions (Hattori *et al.*, 2005; Liang *et al.*, 2007; Biel *et al.*, 2008), effect of Si has rarely been investigated on growth of medicinal plants such as fennel under water stress. It is hypothesized that Si application may improve water stress tolerance and growth of fennel by modulating associated morphophysiological changes. The purpose of this study was to investigate the effects of Si application on growth characteristics, quantitative parameters (seed yield and essential oil percentage), amount of osmotic regulator (proline and soluble sugars) and concentration of some minerals in seeds of fennel under water stress conditions.

MATERIALS AND METHODS

Site Description: Field experiment were carried out during 2014 on agricultural experimental farm of Zabol University, located in Chahnimeh (61°29′ N, 31°23′E, 498 m above sea level), in south east of Iran. The experiment was established in a clay loam soil. The experimental site is located in warm and arid region with mean annual precipitation of 58.9 mm and annual mean long-term average temperature of 22 °C. Table 1 indicates some physiochemical characteristics of the soil.

Table 1. Physicochemical characteristics of soil

Soil texture	Organic matter (%)	Available nutrients (ppm)						EC ($ds.m^{-1}$)	pH
		Fe	Mn	Zn	P	K	N		
Clay loam	0.54	0.8	1.6	1.8	11	124	5.6	2.1	7.8

Experimental scheme: Seedbed preparation comprised ploughing, harrowing and cultivation. Fennel utilized in this study was landrace of Zabol. The experimental design for this study was a split plot randomized complete block design with three replications. Main-plot treatments were irrigation at 90%, 75% and 60% of FC (accordingly no stress, moderate stress and severe stress, respectively). Sub-plot treatments consisted of Si spraying at 5 levels (0, 2.5, 5, 7.5 and 10 mM). Spraying were applied at two stages; the mid-vegetative growth stage and early flowering stage. Water stress was employed at 6 to 8 leaf stage. The treatments were laid-out in 2×3 m plots. The plants were seeded at distance of 0.40 m among rows and 0.30 m within rows on 29th February, 2014. Adjoining sub-plots were dispart by a 0.5 m wide border, and the main-plots were dispart by a 1.5 m wide border.

Water content of soil was monitored manually in all irrigation regimes twice a week before irrigation with TDR[1] using vertically mounted probes. During the plant growth plots were weeded manually. No critical outbreak of disease or insect was observed and no fungicide or pesticide was utilizedto plants.

Plant sampling and analysis: At the end of the growth, five plants were sampled and several vegetative growth characteristics (including LAI, plant height, and umbel number per plant) as well as one-thousand seed weight, concentration of proline, soluble sugar and Si in leaf and concentration of sodium (Na), potassium (K) and calcium (Ca) in seeds were separately noted. At maturity, plants were harvested on July 18, sun-dried for about 2weeks to around 8% water content, threshed and weighed to specified seed yield.

To obtain essential oils, the fresh aerial parts of plants (30-40 g) were subjected to hydro distillation for 2 h. The distillate was dried over anhydrous sodium sulphate and reserved at 4±6 °C. The leaf area index (LAI) was determined as the basis on specific leaf area ($cm\ g^{-1}$) and total leaf dry matter production on a sample of plants. Harvested samples of seeds and leaves oven dried at 75 °C for 72 h and were ground to powder. A quantity of 0.5 g of each sample was digested with 10 N HNO_3, and a flame photometer (model, JENWAYPFP7) was utilized for assessing Na and K contents. Calcium concentration in seed samples were determinedusing spectrophotometry (model, UV-2100 Unico). Si content was determined by ammonium molybdate spectrophotometric (Elliot and Snyder, 1991). Proline colorimetric measuringconducted according to Bates *et al.* (1973) based on proline's reaction with ninhydrin. For proline colorimetric measuring, 1:1:1 proline solution, glacial acetic acid and ninhydrin acid was stored at 100 ºC for 1 hour.

Chlorophyll content was determined by the chlorophyll content meter device (Hansatech Instruments-model-Cl-01, Tokyo, Japan). Soluble carbohydrate content wasmeasured using the phenol-sulfuric acid method (Irrigoyen *et al*., 1992).

Statistics: Data were subjected to analysis of variance. Test of significance difference of the treatment was conducted on a basis of a *t*-test. The significant differences between treatments were compared with the critical difference at a 5 percent level of probability.

RESULTS

Vegetative growth: LAI and plant height differed among different irrigation treatments. In this study, plants grown under no stress conditions produced the largest growth parameters (LAI: 1.41±0.24 and height: 59.64 ±9.34 cm). Plants raised under no stress had 64% greater LAI and 50% greater height compared to those under severe stress (Table 2).

Among Si spray treatments, the least growth characteristicswas obtained in the plants sprayed with tap water. Spraying with 7.5 mM of Si contributed to a higher plant growth parameters compared to the other spray treatments. The highest growth characteristics (LAI: 1.27± 0.20 and height: 54.75 ± 6.14 cm and) were observed with 7.5 mM of Si foliar spray, whereas the

[1]Time Domain Reflectometry

lowest (LAI: 1.06±0.23 and height: 47.03 ± 7.57 cm) growth parameters were obtained with the control (tap water spraying).

Significant interaction between irrigation treatments and Si spraying has been found to exist on the LAI (Table 3). In the partition of this interaction, it was obvious that under severe water stress sprayed plants had significantly greater growth rates compared to those sprayed with tap water.

Table 2. Mean comparison and analysis of variance for growth traits of fennel in different water stress and silicon spraying.

Treatment	Leaf Area Index	Plant height (cm)	Seed yield ($kg.ha^{-1}$)	Essential oil percentage (%)	Essential oil yield ($lit.ha^{-1}$)
Water stress					
No stress	1.41a	59.64a	843.23a	1.47b	12.63b
Mild stress	1.22b	53.85a	739.25b	2.17a	16.23a
Severe stress	0.86c	39.73b	476.42c	1.86a	9.00c
Silicon mM					
0	1.06b	47.03b	555.02c	1.68dc	9.18b
2.5	1.05b	47.35b	583.60c	1.61d	8.90b
5	1.26a	52.92ba	754.50ba	2.11a	15.70a
7.5	1.27a	54.75a	805.72a	1.91ba	15.61a
10	1.16ba	53.31ba	732.65b	1.86bc	13.69a
S.O.V					
Stress	**	**	**	**	**
Silicon	**	*	**	**	**
Stress × Silicon	*	ns	**	*	ns
CV (%)	10.15	12.25	7.93	12.44	17.79

Within each column and each factor, means followed by the same letter do not differ significantly different at the 5% level of probability according to DMRT.
ns, * and ** represent not significant, significant difference over control at the 1 and 5% probability levels.

Yield attributes: Seed yield of plants were significantly influenced by irrigation treatments (Table 2). Seed yield for the plants irrigated at 90% FC (control) was77% greater, than the yields of the plants irrigated at 60% FC. The seed yield of the plants irrigated at 75% FC was also 12% lesser than the control.

Si foliar spraying at rate of 7.5mM increased the seed yields by 45% compared with those of the control. Significant interaction between irrigation and Si spray treatments has been found to exist for seed yield (Fig. 1). Irrigation at 90% FC with Si foliar spraying at 5 and 7.5 mM and irrigation at 60% FC along with tap water foliar spraying produced the highest and least seed yields, respectively.

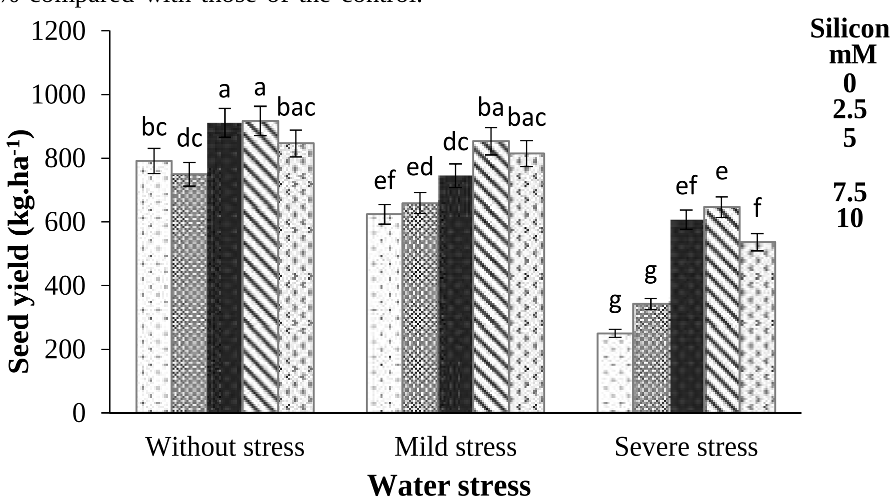

Fig 1- Interaction effects of water stress and silicon spraying on seed yield of fennel.

Values followed by the same letter do not differ significantly at the 5% level of probability according to DMRT.

Yield and percentage of essential oil: There were significant differences among different irrigation treatments in the yield and percentage of essential oil. Mild water stress significantly increased yield and percentage of essential oil compared with the control (no water stress) and severe water stress.

Si spray treatments exhibited rather remarkable differences in the yield and percentage of essential oil (Table 2). Si foliar spraying at concentration of 5 mM increased the yield and percentage of essential oil of fennel by 42 and 20%, respectively, over those of the control. Significant interaction between the irrigation treatments and foliar spray treatments on essential oil percentage was observed (Table 3).

Osmoregulators concentration in leaves: Concentration of proline and soluble carbohydrates differed among various irrigation treatments. In this study, irrigation at 60% FC (severe stress) produced the greatest concentration of osmoregulators (proline: 1.96 ±0.11mg. g^{-1} of fresh weight and soluble carbohydrates: 2.61 ±0.13mg. g^{-1} of dry weight). Plants grown under severe water stress had 19% greater proline and 5% greater soluble carbohydrates compared to those raised at no water stress.

Among the spray treatments, the least concentration of osmoregulators was obtained in the plants sprayed with tap water. Si foliar spray at concentration of 10 mM contributed to a greater concentration of osmoregulators compared to the other spray treatments. The highest concentration of osmoregulators (proline: 1.95 ±0.12 mg. g^{-1} of fresh weight and soluble carbohydrates: 2.47 ±0.14 mg. g^{-1} of dry weight) were obtained with the highest concentration of Si spray, whereas the lowest (proline: 1.48 ±0.10 mg. g^{-1} of fresh weight and soluble carbohydrates: 2.16 ±0.17 mg. g^{-1} of dry weight) concentration of osmoregulators were observed at the control (tap water spraying).

Nutrient concentration in plants: Higher concentrations of K and Ca in seeds were observed in fennel plants grown on plots irrigated at 90% FC (no water stress); the constant trend in K and Ca concentrations in decreasing order was as follows: no water stress, mild water stress, and sever water stress (Table 3).

The concentrations of the above mentioned nutrients in the plants differed significantly because of Si spraying. Na concentration in seeds increased with increasing concentration of Si spraying, and the greatest concentration of this nutrient was obtained in plants sprayed at 10 mM of Si. Plants sprayed with highest concentration of Si (10 mM), on the other hands, had the lowest concentrations of Ca and Na in seeds, whereas the plants sprayed with tap water showed the highest concentrations of these nutrients. Concentrations of Si in leaves were strongly greater in the plants that received Si as foliar applications. No statistically significant difference was observed between the interaction of irrigation treatments and Si foliar spray treatments on nutrient concentrations (Table 4).

Table 3- Mean comparison and **analysis of variance for osmoregulators and some minerals of fennel in different drought stress and silicon spraying**

Treatment	Proline (mg.g^{-1} fresh W)	soluble carbohydrate (mg.g^{-1} DW)	Seed Potassium	Seed Calcium	Seed Sodium	Leaf Si (mg.g^{-1} DW)
				ppm		
Water stress						
No stress	1.35c	2.09c	100.42a	105.34a	38.02b	2.25c
Mild stress	1.72b	2.20b	93.98b	96.5a	42.18b	2.66b
Severe stress	1.96a	2.61a	83.74c	71.74b	49.95a	3.83a
Silicon mM						
0	1.48c	2.16c	88.44c	99.11a	48.02a	1.39c
2.5	1.54c	2.21bc	90.10bc	98.40a	45.27ba	2.67c
5	1.61bc	2.28bac	92.92bac	96.11a	42.87ba	3.00b
7.5	1.81ba	2.39ba	95.4ba	80.52b	41.91ba	3.19ba
10	1.95a	2.47a	96.71a	77.51b	38.85b	3.31a
S.O.V						
Stress	**	**	**	**	**	**
Silicon	**	**	*	*	*	**
Stress × Silicon	*	*	ns	ns	ns	ns
CV (%)	8.16	13.39	6.62	17.19	14.15	10.26

Within each column and each factor, means followed by the same letter do not differ significantly different at the 5% level of probability according to DMRT.
ns, * and ** represent not significant, significant difference over control at the 1 and 5% probability levels.

Table 4- Interaction effect of water stress and silicon spraying on traits of fennel

drought stress	Silicon mM	Leaf Area Index	Proline (mg.g^{-1} fresh W)	soluble carbohydrate (mg.g^{-1} DW)	Percentage of essence
No stress	0	1.44a	1.21d	2.11de	1.34ef
	2.5	1.37a	1.51dc	1.90e	1.10f
	5	1.44a	1.20d	2.11de	1.76edc
	7.5	1.39a	1.40dc	2.17de	1.68edc
	10	1.40a	1.43dc	2.16de	1.49edf
Mild stress	0	1.09dc	1.44dc	2.03de	2.04bac
	2.5	1.05d	1.28d	2.38dc	1.79dc
	5	1.35a	1.81bc	2.24de	2.27ba
	7.5	1.31ba	1.99ba	2.26dc	2.42a
	10	1.28bac	2.09ba	2.10de	2.32ba
Severe stress	0	0.66f	1.80bc	2.33dc	1.66edc
	2.5	0.75f	1.84bc	2.35dc	1.94bdc
	5	1.00ed	1.81bc	2.81ba	2.30ba
	7.5	1.11bdc	2.05ba	2.99a	1.65edc
	10	0.80ef	2.33a	2.58bc	1.76edc

Within each column and each factor, means followed by the same letter do not differ significantly different at the 5% level of probability according to DMRT.

DISCUSSION

One of the first symptoms of water-stressed plants is the loss of turgidity and reduced growth and development ofcells. Many plant process are affected by water stress, with cell growth probably the most sensitive. That is the reason for visible effect of water defection on plant height and LAI (Good and Zaplachiniski, 1994). Reduction in plant height, LAI and number of umbels per plant under water stress could be attributed greatly to photosynthesis impairment and decline in photosynthetic products to transmit to the growing parts of plant. These finding partly agrees with the results of Moosavi *et al.* (2014) who reported that water stress reduces plant height, LAI and number of umbels per plant in fennel.

The results indicated Si Application like the study of Mohaghegh *et al.* (2010) increased plant height and LAI. It is proven that Si through modification of plant water relation, stimulates cell division and cell elongation (Na and Jiashu, 2001) boosts plant immune system(Liang *et al*., 2007) and enhances plant growth.The sharp decline in seed yield under water stress could be attributed to the negative impact of water stress on growth and yield attributes, because stomatal closure and reduced turgidity in water deficit conditions led to reduce growth and seed yield.These results agree with findings of Moosavi *et al.* (2014) in fennel.

Si spraying enhanced seed yield under water stress compared with the control. Increasing seed yield caused by Si application could be due to increasing leaf chlorophyll content, yield attributes and photosynthetically active area (Na and Jiashu, 2001). A positive impact of Si on crop yield under water deficit has been reported by Silva *et al.,* (2012). Si Application by reducing transpiration caused water tolerance (Karmollachaab *et al.*, 2014).

It has been reported that increasing essential oil percentage in Apiaceae family is a mechanism to adapt the plant to water stress (Moosavi *et al*., 2014). It is noteworthy that always essential oil cannot be increased along with increasing in water stress, because assimilates produce osmoticregulators such as soluble sugars and proline in severe water stress (Munns and Tester, 2008). Finding of this experiment showed that severe stress significantly decreased essential oil content over the control. This reduction mainly related to decrease in seed yield. Letchamo *et al.* (1999) concluded that biosynthesis of medicinal plants secondary metabolites positively related to the photosynthesis and negatively relationship to the respiration.

Proline accumulation has a positive relationship with increased resistance to water stress (Saneoka *et al.*, 2004). In this experiment also water stress increasing, increased concentration of proline. There were similar reports about the impact of water stress on wheat (Nayyar, 2003) and nigella (Rezapor *et al*., 2011). In this experiment, Si through increasing proline concentration increased osmotic adjustment during the water stress. There are similar reports regarding increasing in proline content following Si application in barley (Haddad and Moshiri, 2008) and borage (Gagoonani *et al*., 2011).

In water stress and saline conditions, accumulation of soluble carbohydrates play an important role in osmoregulation, turgidity and stability of biomolecules and membranes. In addition, soluble carbohydrates help to reduce cell water potential and cell turgidity under water deficit (Farahat *et al*., 2007). The results of this experiment revealed that soluble carbohydrates content significantly increased under water stress. This results agree with other reports in tarragon (Lotfi *et al*., 2014) and nigella (Rezapor *et al*., 2011). Also, Si increased the soluble carbohydrates. It seems that Si by increasing chlorophyll content, reeducation of oxidative stress, protection of cell and chloroplast membranes and protection of macromolecules such as proteins enhanced the soluble carbohydrates in plants. These stimulates contribute to osmotic adjustment in plants (Silva *et al*., 2012). These findings were in line with report of Saadatmand and Entshari (2013) regarding increasing of soluble carbohydrates in borage under salinity stress and Si application. Water stress increased Na concentration in seeds by 24% in comparison with the control. Na increasing in water stress is a defense mechanism which able plants to adjust the osmotic pressure of cells (Munns and Tester, 2008). Increase in Na of fennel plant is in accordance with the results of experiment on salvia (Sodaeizadeh and Mansour, 2014) and broad bean (El-Tayeb, 2006).Si Application decreased Na in fennel over the control. Based the results of Marschner (1995) Na absorbed passively. Siplays a key rolein strengthof thepore cell wallsby creatingcomplexes with cell wall compounds. Therefore, Si reduces the plantstranspirationand this led to decline insodium absorption (Liang *et al*., 2007).

Water stress decreased seed K by 17% over the control. Liang *et al.* (2007) expressed uptake and transfer of K needs to ATP consumption. Therefore, the concentration of thisnutrient in theplantreduced due to the reductionin theamountof ATP inwater stress. In addition, reducedroot growthunder water stress is thereasons for the decrease in absorption ofthiselementfrom the soilbyplant (Hu *et al*., 2007). Si Application increased seed K. It has been reported that Si under water stressincreases activity of H ±ATPase in the plasma membrane of root cell. Therefore, Si increases absorption and transmission of potassium in plants and increases potassium concentration in plants (Kaya *et al*., 2006).

Ca Concentration under water stress decreased compared with the control. This reduction was attributed to antagonisticrelationofNa and Ca (Ramezani*et al.*, 2011). Plant responses to absorption of Ca under water stress condition depends on soil physicochemical properties, however in most plants along with increasing water stress accumulation and transportation of Ca decreased due to increase in the Na concentration. Si Application imperceptibly decreased Ca seed. Ca movement in plants largely depends on transpiration rate and Si through decrease in transpiration, decreases plant Ca.

Increasing water stress and Si spraying increased Si in leaves. Results of other study conducted on wheat confirmed our findings (Tuna *et al*., 2008).

Conclusion: It may be concluded that Si by affected some physiological activities, amounts of nutrients and increased osmoregulators hence improved tolerance against water stress and growth in fennel plant. The positive effect of Si on crop yield was more evident under water deficit. In medicinal plants, mild water stress is desirable, because it not only increases essential oil percentage, but also do not have a large negative impact on growth and physiological characteristics of fennel.

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